Remarks

Claims 1-101 currently are pending in the present application, however claims 2, 12, 20, 25-27, 29-32 and 34-101 are withdrawn. Claims 1, 3-11, 13-19, 21-24, 28 and 33 have been examined and stand rejected in the pending Office Action dated December 3, 2004. Claims 1, 3, 5, 16-17 and 21-23 are amended herein. Claims 4 and 10-11 are canceled. Support for the amendments can be found throughout the application, and particularly at paragraph 54, which discusses a library of variant peptides, including the amino acid (primary) sequence thereof; paragraphs 67, 86 and 97, which discuss G protein-G protein coupled receptor interaction; paragraph 97, which refers to the in vitro nature of the assays; and paragraphs 49 and 54, which discuss the Gα subunit carboxyl terminus.

Applicants note with appreciation that the objections to the inventors' declaration and the specification have been withdrawn.

Claims 1, 3-11, 13-19, 21-24, 28 and 33 are rejected under 35 U.S.C. §112, first paragraph, as not enabled. The Office Action states that the specification enables a method using a biased peptide library derived from the carboxyl terminus of the "Gα-coupled receptor," but not "all types of G-protein coupled receptor (GPCR) for the peptide library or candidate compounds." The Office Action cites reasons as advanced in an Office Action dated April 17, 2003. Since there is no Office Action of that date in the present application, Applicants assume the Action dated June 11, 2003 was meant to be cited and will reply based on the reasons given on pages 5-9 of this Office Action.

As an initial matter, Applicants would like to point out that there is no "Gα-coupled receptor" recited in the specification or claims of this application, or known to Applicants. The G protein comprises a Gα subunit, however there

is no specific Gα-coupled GPCR that is coupled to Gα distinct from G proteins, nor is Gα a type of GPCR. Gα is not a type of G protein or a type of G protein coupled receptor, but rather a subunit common to all G proteins. The Office Action also seems to assume that G proteins are GPCR ligands. This also is not true; ligands and G proteins bind to GPCRs at different sites, now referred to as orthosteric and allosteric sites, respectively. Because at least some of the bases for rejection of the pending claims appear to relate to this type of misunderstanding concerning the molecules of the invention, Applicants have amended the claims for the sake of clarity and to assist the reader in understanding the molecules involved and their relationship to one another. Therefore, the term G protein coupled receptor has been replaced with the acronym GPCR in the claims to reduce repetition of the phrase "G protein" and reduce the potential for confusion between G protein coupled receptor and G protein.

In the Office Action dated June 11, 2003, The Office states the opinion that the specification enables using a peptide library derived from the carboxyl terminus of the Gα-coupled receptor but does not enable using a peptide library derived from any or all GPCR. See Office Action, page 5, lines 7-12. The claims presented here do not involve a library derived from any receptor; not from any GPCR nor from any "Gα-coupled receptor." The claims recite providing a peptide library which is based on a native G protein peptide, not a G protein coupled receptor (GPCR) peptide. Therefore, the Office's stated reasoning is specious because: (1) there is no Gα-coupled receptor; (2) the peptide libraries recited in the claims are not derived from GPCR or any species of GPCR.

The Office opines that the claimed scope is infinite or undefined because the Examples disclose only methods using a

"biased peptide library from the carboxyl-end of the GPCR" while the claims apparently are interpreted by the Office as drawn to methods using libraries based on a wide range of biologically active receptors. See Office Action, page 5, lines 19-24. The libraries discussed in the Examples are not based on the carboxyl-end of the GPCR but "on the carboxyl terminal sequence of Gαt (IKENLKDCGLF; SEQ ID NO:139)," for example. See specification page 49, paragraph 100. Gαt is a species of Gα subunit, which is a subunit of G proteins. The subunits of the G protein, including Gα, are discussed in the specification at paragraph 3; Gt is listed in Table II as a species of Gα. Therefore, it is clear from this disclosure and the specification generally, that Example 1 refers to a library based on a sequence taken from a G protein subunit and not a GPCR.

The Office then criticizes the assay described in the application because it is able to discriminate among analogs that have different levels of binding, from no effect to significant effect, and because the binding of G protein to GPCR is specific. The Office seems to equate the discrimination of the assay with unpredictability. This logical connection does not hold. The screen provided by the Applicants here enables the user to determine whether a given compound specifically binds to a GPCR, thus eliminating any perceived "unpredictability" in determining what compounds bind. The Office is arguing that the assay's results are unpredictable because it cannot be known in advance of performing the assay whether a difference in one amino acid affects binding of a G protein peptide to its receptor (i.e. some analogs tested had significant effects and some did not). It is the nature of screening assays, as is well-known in the art, that some compounds are positive and many are not. If the results were predictable in advance of performing the screen, there would be no need to perform the screen or any screen.

In effect, the Office is arguing that because the screen provides an answer to an otherwise unpredictable question, the assay itself is unpredictable. If such were the case, no screening assay would be useful. For example, a screen for HIV in blood would be considered unpredictable and not enabled unless either all blood samples contained HIV or the user could pre-select only positive samples for testing. Applicants submit that such a test would be useful, workable and enabled even if no blood samples provided positive results because the purpose of the assay is to determine whether the sample contains HIV or not. The screening assay here is performed to determine whether a peptide binds to the intracellular face of GPCR and to reliably detect when a peptide does bind and when it does not bind. If a peptide does not bind, this does not indicate that the assay has failed or that the assay is unpredictable; it indicates that the particular peptide does not bind, which is what the screen is designed to determine.

If all peptides (or even most peptides or many peptides) tested bound to the GPCR, this would indeed cast suspicion on the value of the assay. It is the nature of combinatorial chemistry-type assays, which are used routinely in drug discovery programs, that literally 10^5 compounds or more are tested to find one or a few that bind strongly. See references cited below and provided with the Information Disclosure Statement, which refer to billions of compounds. This is not indicative of unpredictability of the method of screening, but of the rare nature of specific and appropriate binding. The Office should not penalize Applicants because they have invented a method to screen more reliably for an event that is rare or that could not previously be predicted. These qualities emphasize the enormous value of the screening method, not that the assay requires undue experimentation.

The predictability of an assay must be judged according to standards in the art to which it belongs. In the drug discovery art, practitioners routinely screen millions of compounds and obtain positive results in the tens or hundreds, many of which are false positives or only weak responders. One of the many advantages of this assay is that false positives and identification of weak responders is reduced. As a result, the total number of compounds identified from a high throughput screen of a million compounds may be smaller, however this is a benefit of the assay and not a signal that it is not functioning or enabled--the assay identifies only compounds that are stronger binders. Applicants therefore submit that the claimed screening methods are not unpredictable but, to the contrary, increase the predictability of results when performing these types of compound screens. In any case, several exemplary screens were performed and each identified one or more compounds.

The Office states that the specification does not provide sufficient guidance such that a reader can determine which G proteins "can be made into a library." June 11, 2002 Office Action, page 7, lines 7-12. Applicants would like to call the Office's attention to paragraphs 52-55 of the specification, which describe the nature of the G protein/GPCR interactions useful for screening methods and providing a number of examples. In addition, Table III on page 24, entitled "Exemplary Native G Protein Sequences for Library or Minigene Construction," provides several dozen specific examples of different G proteins (including some exemplary peptide sequences) suitable on which to base library construction. To state that the specification lacks guidance as to which G proteins on which one should base the library is simply unreasonable.

The Office next states that the specification does not provide working examples for any type of G proteins, their

receptors and methods of making libraries and an expression vehicle that can "accept a G-protein ligand" without deleterious effects to the vectors and/or hosts. First of all, construction of peptide libraries per se, and peptide display libraries, is not new in the art. This type of construction was not considered unreliable or unpredictable in its function at the time this application was filed. See, for example, Kay et al., Methods 24:240-246, 2001; Dani, J. Receptor Sign. Transduct. Res. 20(4):469-488, 2001; Rodi and Makowski, Curr. Opin. Biotechnol. 10:87-93, 1999; Gilchrist et al., Meth. Enzymol. 315:388-404, 2000; Schatz et al, Meth. Enzymol. These references are listed in an Information Disclosure Statement which accompanies this response. These references all bear dates before or near this application's filing date and describe methods for making and using peptide libraries. The concerns articulated in the Office Action were known in the art. Various methods were in use to overcome them. Therefore, the art enables the use of peptide libraries. What is known in the art is not necessary to include in the specification for enablement of the claimed invention and preferably is omitted from the specification of a patent application. M.P.E.P. §2164.01.

Second, Examples 1 and 2 alone provide a working example showing successful construction of 6 different libraries with greater than 10^9 independent clones per microgram of vector each. Applicants submit that the creation of several different libraries, each containing 10^9 independent clones is more than sufficient to demonstrate that the procedures function with large numbers of peptides. Examples 7-9 demonstrate that the libraries can be used in "panning" to identify strong binders. Applicants submit that this working example, along with the discussion in the specification and what is known in the art, provides more

than sufficient guidance to the skilled reader as to how to make these types of libraries.

Whether the libraries can "accept a G-protein ligand" is not relevant, since the methods which are claimed here do not relate in any way to "G protein ligands," which are not defined in the Office Action and not mentioned in the specification or claims of this application. Applicants do not understand this term. The inventive screens do not relate to "ligands" that bind either G proteins or GPCR. Moreover, a G protein is not a ligand. Ligand binding is discussed as part of the background material describing how G proteins operate, to distinguish the present invention, which does not relate to assays for extracellular ligands, and to provide a method of assaying to determine and measure the activation of a GPCR. The screens of this invention do not relate to discovery of traditional GPCR ligands or to any "G protein ligands."

Next, the Office remarks that the claims broadly encompass a "large diversity of expression vectors, nucleic acids encoding peptides, peptide combinatorial libraries, candidate compound library, G-protein ligand binding receptor and receptors," which renders it difficult to determine the expression levels of the peptide sequences or to recognize whether or not a specific cell is expressing "a member of the insert." The Office seems to be concerned that expression of the library may not be efficient or detectable in particular cells, rendering the methods experimental and therefore not enabled.

These types of peptide expression systems are not new to the art, and as discussed above are not necessary to be described in minute detail to enable the claims pending here. The methods described here for presentation of peptides were successful with high transformation efficiencies. See specification at paragraphs 58-59. The performance of individual cells in the

expression system is not relevant since individual cells are not assayed separately. The library is provided in the form of a bacterial lysate or a purified fusion protein, for example, and not as a single cell or clone of cells. See paragraphs 115 and 64 of the specification. Moreover, these methods are well-accepted to function as they are described in the present application to present and display any peptide, for example the many peptides screened in the examples provided here and in the art. See, for example, references cited above and Cheadle et al., J. Biol. Chem. 269(39):24034-24039, 1994; Cwirla et al., Science 276:1696-1699, 1997; Martin et al., J. Biol. Chem. 271(1):361-366, 1996.

The Office also remarks that "expression of determinants on the surface" exist only for phages and that phage vectors could cause protein domains that do not fold due to disulfide bonds. It is unclear why the Office is concerned about these factors, which are not of concern to skilled practitioners. With respect to the libraries, a surface location of the protein on cells is not claimed and not relevant to the methods. While it is true that disulfide bonds may affect protein folding, this is of less concern the shorter the peptides are. What is more, the prior art discussed this factor, which has not been a major concern when using these libraries. See references cited earlier. Applicants refer the Office to discussion above concerning the efficiency and usefulness of the approaches described in the specification for peptide libraries.

With respect to GPCRs, the GPCR is a surface (transmembrane) protein. The specification clearly provides methods for their isolation from natural sources or after expression in a recombinant cell, or synthesis, and methods for their reconstitution in vesicles. The expression of GPCR is completely independent from expression of peptide libraries, and is not

claimed as a novel aspect of this invention. The prior art, along with the instruction and guidance in the specification are more than sufficient to aid the reader in preparing GPCR for assay by expression from a recombinant cell at high levels in a variety of heterologous systems. See, for example, Windh et al., J. Biol. Chem. 274(39):27351-27358, 1999; Francken et al., Mol. Pharmacol. 57:1034-1044, 2000; Glass and Northup, Mol. Pharmacol. 56:1362-1369, 1999. Applicants respectfully submit that expression of "determinants" on a surface is not relevant to GPCR expression and is not encompassed by the claims pending here and therefore that this factor is not relevant to their patentability. Applicants have recited peptide libraries and compound libraries in their claims. If it is desired to do so, libraries are known to effectively present peptides for screening. See references cited above. Further, they have been described in the specification with more than enough detail and breadth to enable any skilled worker in this field to understand what they are and how to use them.

The Office Action states that the art is inherently unpredictable because insertion of a foreign sequence into a protein may have unpredictable effects on the protein or its expression, thus likely perturbing the function and stability of the fusion. This criticism is again merely a repeated statement that the Office doubts the workability of the display libraries taught for use in some embodiments of the present invention. Applicants and others have routinely used these library expression methods. See citations above relevant to this subject matter and Zwick et al., Curr. Opin. Biotchnol. 9:427-436, 1998; Koivunen et al., J. Nucl. Med. 40:883-888, 1999. Completely functional libraries are reported in the specification, along with the screen results of several working examples. Applicants refer again to Examples 1 and 2, which show successful

construction of 6 different libraries with greater than 10^9 independent clones per microgram of vector each. The methods work, and they have been demonstrated to have worked with several different libraries containing 6×10^9 independent clones based on several different peptide sequences.

The Office has provided no reasoning why the skilled person would not be able to repeat the types of assays reported in the specification using any suitable, known library or would believe that the methods do not work as disclosed. There would be any doubt in the mind of the skilled reader that these methods would have been considered routine given the art and the discussion and examples provided by Applicants. The guidance in the application as filed is more than sufficient to demonstrate that the procedures for peptide display libraries function with large numbers of peptides and with, for example, different G protein peptides. Applicants therefore submit that merely to state that the assay is unpredictable does not make it so. The Office has provided no meaningful reasoning as to why the skilled person would doubt the workability of these assays. The unsupported conclusions given in the Office Action are even less reasonable since the specification provides several working examples showing that the display libraries do function and do enable the assayer to identify compounds that bind GPCR as claimed.

The Office has provided additional responses in the currently pending Office Action. The Office remarks that "if a compound has no definite or given structure," screening cannot be done because there will not be assurance that a high affinity compound will be identified. First, this remark ignores the fact that screens of huge numbers of compounds of random or unidentified structure are performed on a daily and routine basis by researchers and drug companies. This is the nature of combinatorial chemistry assays. <u>All</u> combinatorial chemistry

methods screen very large numbers of compounds with no pre-identified assurance that a blockbuster drug will be discovered in the screen. Predicatability is measured according to what is usual in a particular art. In this art it is usual to screen random libraries of compounds. This assay is better than the assays currently being used on a daily basis to screen similar libraries because it assures fewer false positives and greater predictability of the results than prior assays, not less.

Second, the specification clearly shows that the assay works. The application as filed assayed several different libraries based on sequences from several different G proteins and in each case one or more high affinity binder was identified. Once the high affinity binder has been identified, it is a matter of routine for the combinatorial chemist to use this identified high affinity binder to screen any library with any structure, defined or random, as they do in their daily work. Such screens are the bread and butter of combinatorial chemists, who screen millions of compounds routinely.

The test of enablement is not whether experimentation is necessary, but whether whatever experimentation that is necessary is undue. M.P.E.P. §6164.01. Applicants submit that no experimentation is necessary to perform the assays provided by this invention. The Office has erroneously termed the assay not enabled simply because a large number of molecules are screened and the results that the screen will provide are not known prior to performing the screen. However, this is the way this screen and all combinatorial chemistry-type screens are performed. Even if performing the screen itself is deemed "experimentation" at all, which Applicants maintain it is not (since there is ample guidance on how to perform the claimed assays), performing such screens are routine in the art and therefore cannot be "undue" experimentation.

The Office Action states that exactly how a compound library can be based on a cognate G protein is not positively recited in the claims. Applicants have amended the claims to recite that it is based on the primary sequence as suggested by the Office. The Office Action then asks if there is one binding site of the cognate G protein. This question is answered both in the prior art and in the specification. There is more than one site involved in GPCR binding. These sites are known. Moreover, there exist in the art assays to identify and/or confirm these sites. Any of these sites may be used with the invention. However, to further prosecution at this time, Applicants have amended the claims to recite that the sequence upon which the library is based is a native G protein Gα subunit carboxyl terminal peptide sequence. Dependent claims 4, 10 and 11 are canceled herein as redundant over the amended claim 1. Applicants reserve the right to continue prosecution of the subject matter canceled from the claims with these amendments.

The Office asks if the binding site is based on deleting, substituting or adding different residues of a cognate G protein. The binding sites are known. In any case, however, the claims are now limited to Gα subunit carboxyl terminal peptide sequences, and so are defined further as to binding sites. If the Office meant to refer to libraries in asking this question, Applicants have amended claim 1 to recite a library of variant peptides. This phrase is supported by the specification as a whole and by paragraphs 54 and 55, which also discusses and describes these libraries.

The Office asks how long a peptide must be to result in a library and what size library produces a compound. Any length of peptide above two amino acid residues may be used to produce a library. As taught in the specification, however, preferred peptides may be about 7-70 amino acids long or about 8-50 amino

acids long or preferably about 9 to about 23 amino acids long and most preferably about 11 amino acids long.

The question as to what size library is needed to produce a compound evidences a fundamental misunderstanding of the routine nature of combinatorial chemistry. Large and indeterminate numbers of compounds in one or more libraries are screened routinely. The screens of these and additional compounds continue until one or a few compounds are identified or until all compounds in the chosen library(ies) have been screened. Any identified compounds are further tested. Additional compounds and additional libraries of compounds are screened and more compounds are identified. The process continues. If no compounds are identified, another library is screened. If a large number of compounds are identified, screening may stop or may continue. Commonly, using prior art screens, many of the compounds identified were false positives; the present invention improves the results by reducing the false positives identified.

The fact that the assays are used with no predefined endpoint does not make the assays unpredictable. As explained above, that is the nature of this type of assay. The specification provides several working examples of libraries which were screened and which identified high affinity binding peptides in every case. These can serve as guidance to the size of a library to screen to the skilled reader and provide confidence that libraries of manageable size can identify compounds as claimed. Applicants submit that the use of the inventive assay according to the routine manner in which these assays are used does not render it unenabled, but quite the contrary.

The Office refers to "other claimed variables" in the assay, for example "inhibitory substance." This term does not appear in the claims, however the preamble identifies the claim as a method

of identifying a GPCR signaling inhibitor. The method steps show how to identify such a signaling inhibitor. Applicants request clarification of the asserted undefined structures of claimed variables to which the Office objects.

The Office states that the "evidence provided in the specification relates to a single based library of definite structure for different G protein coupled receptors." To the contrary, the specification provides results of screening using libraries based on G11, Gt, Gq and Gs for activity related to different GPCR and teaches how to perform assays with dozens of others. Applicants submit that this is ample support to enable these assays, particularly since the sequences of the G proteins are known and the regions of the G proteins that are implicated in GPCR binding are known. Applicants therefore submit that this asserted reasoning and the statements related to the Azpiazu and Blahos references or other prior art on pages 5-6 cannot apply here. In addition, Applicants have amended the claims herein to recite a native G protein Gα subunit carboxyl terminal peptide sequence, which further defines the peptide library recited in step (a) of claim 1.

The Office refers, on page 7, first paragraph, of the present Office Action, to the wide range of extracellular ligands that bind to various GPCR. This wide range of ligand and ligand reactions is asserted to introduce unpredictability to choosing a G protein. As explained previously, the G protein binds to the GPCR on its intracellular face and not on the extracellular ligand-binding domain. The two sites for binding are not related at all with respect to this screen. Because the site to which this technology is directed is separate and distinct from that of the ligand binding site, the ability to predict the reaction(s) between ligand and GPCR is irrelevant and not a proper basis for any asserted unpredictability in the assays of the invention.

GPCR/G protein pairs are known in different cell systems. Therefore it is a matter of routine, given the guidance in the specification, to choose from among known interactions between GPCR and G proteins, or to determine which G proteins can bind a given GPCR, and then screen an appropriate peptide library as described, regardless of what ligands may bind the receptor.

The Office refers to Tables II and III as listing peptides that serve as the basis for appropriate libraries to use in the inventive methods rather than as examples of peptides obtained using the methods. Applicants refer the Office to Tables V and IX-XVIII for peptides identified by the screens of the present invention.

The Office asserts that the assays are performed in vivo because the claims recite an intracellular location for binding. First of all, the term intracellular in claim 1 clearly refers to the location of binding of the native G protein sequence on which the library is based--it does not require the presence of an intracellular compartment during assay. Second, the term "in vivo" refers to inside a living organism. The mere presence of a cell would not render an assay carried out in a plastic tube an "in vivo" assay. A great many assays that involve cells are in vitro assays. Therefore, applicants strongly disagree with this new definition of "in vivo" to apparently include all experimentation in systems that test binding to an intra- or extra-cellular binding site. Nevertheless, to avoid any possible confusion on the part of the Office, Applicants have amended claim 1 to recite "in vitro" in steps (b) and (e), which are screening steps.

The Office asserts that the methods of the invention cannot be operated without a library, vector or expression system. The claims do recite a library (the claims recite "a library of variant peptides based on the primary sequence of a native

G protein Gά subunit carboxyl terminal peptide sequence that binds to said GPCR on a G protein interaction site of said GPCR."). Variant peptide libraries are known in the art and exemplary suitable libraries are described in the specification in great detail. Preferred peptide libraries are peptides-on-plasmids display libraries which generally are known. See cited references. The knowledge of the art combined with the voluminous descriptions in the specification provide more than enough information for the skilled person to duplicate any of the assays using any known G protein sequence on which to base a peptide library. These libraries are not new and are not unpredictable.

The Office asserts that the claims do not recite a particular kind of library or receptor, resulting in a huge claim scope. The claims recite a library of variant peptides based on the primary sequence of a native G protein Gα subunit carboxyl terminal peptide sequence that binds to said GPCR on an intracellular location of said GPCR. Applicants submit that the library is specifically recited and is enabled in its full scope.

The Office refers to an undefined "specific peptide library" which, when used in the method, "unexpectedly fails to produce the expected results for some of the peptides in the library." Applicants do not understand to what specific library the Office is referring because several peptide libraries based on different peptide sequences were disclosed and results were presented from their use, however the <u>expected</u> result for <u>any</u> library screened in a high throughput combinatorial chemistry binding-type assay, including completely random libraries, is that a <u>rare member</u> of the library will strongly bind in the assay. This is borne out by the published art and by the results presented here with several different libraries based on several different G proteins and screened for binding to several different GPCR. The Office

seems to be implying that only if a specific library has been previously shown to contain a member that binds in the assay, is that library enabled for use in the screen. Applicants are not required to specifically test each and every library in order to enable a claim to an assay method that can screen the members of any library.

The Office has not presented any evidence or any reasoning why the assay method claimed here is not capable of screening any group of compounds to determine if they bind to the GPCR. The Office only seems to doubt that any and all libraries definitely will provide a positive result. The nature of combinatorial chemistry/high throughput screening is such that a large number of compounds are screened, with the hope that one or a few will provide a positive result. In routine practice of this art, negative results are obtained with the vast majority of screened compounds and one is never guaranteed that a particular library will yield a positive result. That does not render prior art assays not enabled, nor does it render the present assays not enabled.

In the present amendments, Applicants have added claim language to better define a class of libraries. Because GPCRs all function in the same way with respect to G protein binding and this assay screens for this particular type of binding, any GPCR and any library can be used to test for this particular type of binding. Because GPCR-binding regions of G proteins are known, any library of variants based on these known binding region sequences are preferred as they are more likely to provide the greatest percentage of positive results to identify peptides with high binding affinity.

For the above reasons, and in light of the amendments to the claims, Applicants request reconsideration and withdrawal of the rejection of the claims based on grounds of enablement.

The Office states that the rejections of claims under 35 U.S.C. §112 have been withdrawn. Applicants assume this statement refers to rejections under the second paragraph of this statute section.

Claims 1, 3-11, 13-19, 21-24 and 33 are rejected under 35 U.S.C. §103(a) as obvious over Coughlin et al. or Fowlkes et al. in view of Gilchrist for the reasons set forth in the Office Action of April 17, 2003 [sic, June 11, 2003].

To make out a prima facie case of obviousness against a claim, the Office must meet three criteria: (1) the cited prior art references must teach or suggest each and every element of the rejected claim, (2) there must be motivation to combine or modify what is fairly disclosed in the references to achieve the claimed invention and (3) there must be a reasonable expectation of success. M.P.E.P. §2143. Applicants respectfully submit that the Office cannot make out a prima facie case of obviousness with respect to the presently claimed invention based on the Coughlin et al. or Fowlkes et al. and Gilchrist et al. references.

In the previous substantive Office Action, the Office describes the disclosures of Coughlin et al. as relating to a method for identifying compounds that interact with a cellular receptor using a library of compounds that bind to the receptor at the location an extracellular ligand binds to the receptor. This reference relates to screening for ligand (traditional agonist) binding at PAR3, a GPCR. The teaching of Coughlin et al. does not involve, mention or hint at assaying G protein/ GPCR interactions. Coughlin et al. do not discuss or even suggest methods involving a peptide library based on a native binding sequence in any binding molecule for screening in comparison to the native binding sequence, even for putative extracellular ligands, and does not mention, suggest, or even hint at a method which is capable of discovering molecules that

specifically affect a specific G protein coupled receptor interaction intracellularly. This reference does not teach or suggest a two-step screening method; a method of identifying a GPCR signaling inhibitor; a library of variant peptides based on the primary sequence of a native G protein Gα subunit carboxyl terminal peptide sequence that binds to said GPCR on an intracellular location of said GPCR; an in vitro screen to assay for binding to said GPCR to identify peptide library members that bind to said GPCR with higher affinity than that of said native G protein peptide sequence; selecting a member of said peptide library having binding to said GPCR of higher affinity than that of said native G protein peptide sequence; providing a library of candidate compounds to screen for binding to said GPCR; or screening said library of candidate compounds in vitro for binding to said GPCR in competition with a member of the peptide library selected earlier to identify a member of the library of candidate compounds having binding to the GPCR of equal or higher affinity than that of the peptide selected earlier. All of the above are required limitations of the claims here pending. The reference does not teach any method which can assay for G protein/GPCR binding in any context and lacks even the merest suggestion of the vast majority of claim limitations.

The teachings of Coughlin et al. are confined to traditional ligand binding assays using the PAR3 receptor and do not even relate to the invention claimed here. The present invention does not involve receptor agonists or antagonists that mimic or block a naturally-occurring activating ligand. The primary reference therefore lacks teaching, suggestion or even the merest hint of the assay presented here or even a similar assay. The most Coughlin et al. teach is a recombinant GPCR (PAR3) and a traditional ligand binding assay involving "candidate" agonists and antagonists. Coughlin does not provide any enabling

disclosure with respect to screening of libraries and only briefly mentions this idea in column 2 of the patent, contrary to the Office's description (see June 11, 2003 Office Action which cites columns 2-4 for description of library screening not contained in the reference). There is absolutely no disclosure even related to or which could possibly suggest any assay for binding to GPCR on the intracellular portion where G proteins interact nor of the methods required for the claimed assay, even for the extracellular ligands.

Fowlkes et al. are cited as disclosing "the same method as Coughlin." The Office specifically refers to teaching on page 128 and in the claims. Example 11, on page 128 of the reference, relates to isolation of surrogate ligands for a nuclear hormone receptor and therefore is not even relevant to the subject matter of this application. Fowlkes et al. discusses only receptor ligands and does not relate in any way to screens that assay for binding to G protein interaction sites on a GPCR. Like Coughlin et al., this reference does not contain even the merest hint that would suggest performing the assay claimed here, and does not disclose or suggest the steps of the assay recited in the claims of the present application. Fowlkes et al. does not even mention G protein/GPCR interactions on the intracellular face of the receptor.

Applicants refer the Office to the previous response for detailed discussion of these references. The Office has pointed to no disclosure whatsoever that teaches or suggests the required elements of the claims. Applicants therefore respectfully request that the Office review the claims at this stage, with the present amendments, and compare the disclosures of the references to the required method steps of the claims. Neither of these primary references discuss an assay for identifying molecules that interfere with G protein signaling. They do not teach or

suggest a two-step screening assay. They do not teach, suggest or even hint at a library of variant peptides based on the primary sequence of a native G protein Gα subunit carboxyl terminal peptide sequence that binds to a GPCR on an intracellular location of the GPCR and therefore cannot teach, suggest or even hint at methods that perform steps (b) and (c). There is no disclosure or suggestion of a second screening step at all, much less the step of (e), which recites screening in competition with a library member selected in step (c). The methods of Coughlin et al. and Fowlkes et al. do not result in identification of a GPCR signaling inhibitor. It is clear that not only some but virtually all of the limitations of claim 1 prior to amendment or now pending are not disclosed, suggested or even hinted at in either of the primary references cited here.

The Office has cited Gilchrist et al. to make up for these enormous deficiencies. This reference is cited for teaching on pages 14913-14918, a carboxyl-terminal Gα subunit-based combinatorial peptide library which is screened for binding to adenosine receptors and then in agonist-antagonist competition. This description of the teachings and fair description of the Gilchrist reference is not accurate and is misleading. Gilchrist et al. disclose peptide analogs of Gα peptides and testing of their ability to bind a GPCR and stabilize it in a particular conformation. The "agonist-antagonist competition" assays to which the Office refers are not in any way related to screening of a compound in competition with a Gα subunit-based combinatorial peptide library member selected in a first screening step for binding to a GPCR-G protein interaction site.

The claims here relate to a competitive binding-type assay for the G protein interaction site on a GPCR to identify a compound with equal or higher affinity than that of the native binder. The amended claims clearly recite screening a library of

candidate compounds for binding to the GPCR in competition with a member of the peptide library selected in step (c) to identify a member of said library of candidate compounds having binding to the GPCR. The member selected in (c) was identified as a compound that binds to the G protein interaction site of a GPCR and the second screening step also is for binding at this site.

The assays of Gilchrist et al. involve competition of an agonist and antagonist of the GPCR at the ligand-binding site, not competition at the G protein/GPCR interaction site. The ligands used (cyclohexyl adenosine (CHA)-agonist, (R)-N^6-(phenylisopropyl)adenosine (R-PIA)-agonist and 1,3 dipropyl-8-cyclopentylxanthine (DPCPX)-antagonist) bind at the orthosteric ligand binding site on the receptor. (The claims here do not relate to the orthosteric ligand binding site but rather an allosteric site on the GPCR). As traditional orthosteric ligands, they bind at the extracellular ligand binding site and do not bind at the G protein interaction site of the GPCR. The purpose of these ligand binding studies of Gilchrist et al. was to investigate the effect that the G protein peptide analog had on the receptor as it relates to activation state (high affinity state versus low affinity state), i.e. to determine whether the GPCR was in a high or low affinity state. Traditional ligand binding assays, which use a labeled "probe" or ligand to directly monitor occupancy of the orthosteric site on the receptor, are biased towards detection of orthosteric effects. This is not related to a competition assay for GPCR-G protein interaction site binding.

The Office Action states on page 11, lines 1-3, that Gilchrist "discloses finding (identifying as claimed) the inhibitors for G protein coupled receptors and its ligand, G protein." This statement implies that G protein is the ligand for G protein coupled receptors (GPCR). This is not the case.

At no point in Gilchrist is the G protein identified as a ligand for a GPCR. GPCRs are activated via ligands acting at the orthosteric binding site (on the extracellular face of the plasma membrane). Ligand binding induces conformational changes that subsequently lead to G protein association with the GPCR. Gilchrist demonstrates, in two independent assays (agonist-binding as well as competition assays) that the carboxyl terminal Gα peptide analogs had no effect on the high affinity state of the Adenosine A1 receptor, using two different ligands ([^{125}I]ABA or R-PIA).

In addition, on page 11, lines 5-6, the Office Action states that "Gilchrist screened the library for its inhibitory (antagonist) effect." In fact, no screening was described in the paper; the authors tested six peptide analogs that had been identified in a screen using a different GPCR, rhodopsin, and had been validated as inducing the high affinity state of the receptor. This paper shows that some but not all of the carboxyl terminal Gα peptide analogs inhibited the ability of receptors (Adenosine and $GABA_B$) to signal normally through their G protein, as measured by GIRK. It does not teach the library screening which is claimed and does not teach, suggest, or even hint that the steps (d)-(f) of claim 1 should be performed.

Applicants would like to draw the Office's attention to the description of the "competition" assays on page 14914, first column, first full paragraph. Competition clearly is between the antagonist [^{3}H]DPCPX and increasing concentrations of agonist R-PIA. These compounds are ligands and do not bind at the G protein/GPCR interface, hence they clearly are not competing with the G protein peptide analog. Binding of ligands also was used to estimate the number of GPCR in the membranes tested (page 14914, first column, second full paragraph). The purpose of these assays is described at page 14917, second column, third

full paragraph, which states: "[h]igh and low affinity states of the receptor are detectable in this assay condition." There is no disclosure whatsoever, and no suggestion or hint, that steps (d)-(f) of claim 1 should be performed.

Applicants therefore submit that the disclosures of Gilchrist et al. do not make up for the clear lack of any teaching regarding G protein/GPCR interaction site binding assays. Even assuming that the skilled reader would combine the disparate teachings of these references, there is no suggestion in the combination that would lead any skilled person to perform the assays which are claimed here. First, the combination of all references lacks several required claim elements, most of which relate to the second screening steps of the claim. Second, there is no motivation provided in the art cited or any other art to combine the teachings of the primary references regarding traditional ligand binding assays with the one-step process taught by Gilchrist et al. for testing binding to the G protein/GPCR interaction site and its effects. If the teachings were combined, there still is no motivation provided to perform additional steps required by the claims but not mentioned, suggested or even hinted at in any of the cited references.

Gilchrist et al. does not teach each and every limitation of the pending claims, alone or even in combination with the primary references. The Office discusses disclosure in Gilchrist et al. relating to identification of peptide analogs (Office Action, page 10). Applicants have acknowledged the fact that Gilchrist et al. identified G protein peptide analogs. Gilchrist et al., however, did not teach or disclose each and every limitation of the claims and most particularly did not teach or suggest a two-step assay procedure or any assay procedure that screens a library of candidate compounds for binding to the G protein interaction site of GPCR in competition with a peptide selected

in a first screening step. A rejection under 35 U.S.C. §103 is not proper unless the Office can show teaching or suggestion of each and every claim limitation. The teachings of Coughlin et al. and Fowlkes et al. do not cure the deficiencies of Gilchrist et al. because they also do not contain any teaching or suggestion relating to a two-step assay of this type or of any type.

Applicants submit that the Office has not met even one of the three criteria necessary to make out a prima facie case of obviousness against the claims here, and particularly not the claims as amended. For all the above reasons, Applicants request that the Office reconsider and withdraw the pending rejections on grounds of obviousness.

Respectfully submitted,

By _____

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